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Foliar Penetration of Picloram and 2,4-D in Aspen and Balsam Poplar[1]

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Abstract. Added surfactant (Atlox 210) at 1% (v/v) and high relative humidity enhanced the penetration of both 4-amino-3,5,6-trichloropicolinic acid (picloram) and (2,4-dichlorophenoxy)acetic acid (2,4-D) into detached leaves of aspen poplar (*Populus tremuloides* Michx.). The influence of added surfactant was greater for picloram and the dimethylamine of 2,4-D than for the ethyl or butoxyethanol ester of 2,4-D. Penetration of picloram and the dimethylamine of 2,4-D occurred more readily from the abaxial than from adaxial surfaces of leaves. The ethyl ester of 2,4-D penetrated equally readily from both leaf surfaces. Penetration of picloram and the dimethylamine of 2,4-D from the adaxial surface of leaves occurred readily in young leaves in early June. There was an increase in penetration in early July followed by a decrease in August and September to a level equal to or less than that in June. Penetration from the abaxial surface of leaves was nearly equal in June and July, but there was a gradual decrease in August and September. An increase in temperature from 10 to 25.5 or to 40.5 C resulted in a sharp increase in penetration of both picloram and 2,4-D under both low and high relative humidity. Autoradiographic evidence showed that movement of picloram within the leaf also was much more extensive at the higher temperatures. Partial removal of cuticular waxes from the adaxial surface of leaves with chloroform resulted in up to four-fold increases in penetration of picloram and 2,4-D. Differences in penetration rate of picloram between leaves of aspen poplar and balsam poplar (*Populus balsamifera* L.) did not account for reported differences in susceptibility between these two species.

INTRODUCTION

VARIOUS derivatives of (2,4-dichlorophenoxy)acetic acid (2,4-D) have been used for many years with varying success to control a number of woody plants (4, 10, 11). In recent years, 4-amino-3,5,6-trichloropicolinic acid (picloram) has been effective for the control of 2,4-D-resistant woody species (4, 13, 25). Differences in susceptibility of some species to picloram, for example, aspen poplar (*Populus tremuloides* Michx.) and balsam poplar (*Populus balsamifera* L.) (4), have not yet been fully explained. Furthermore, reports of marked increases in effectiveness of picloram following the addition of a specific surfactant (4, 12) led to the suggestion that penetration into leaves of some of these woody species may be limiting the herbicide's effectiveness.

It has long been recognized that added surfactants may facilitate the emulsifying, spreading, wetting, solubilizing, and/or surface-modifying properties of herbicide formulations and may bring about enhancement of penetration and herbicidal action (2, 16, 21, 23). Considerable evidence has been accumulated to show that high relative humidity in the atmosphere around the leaf enhances the penetration of foliar applied herbicides and growth regulators such as 2,4-D, triethanolamine benzoate (benzoic acid) (19), and 2,2-dichloropropionic acid (dalapon) (20), but no such information has been reported regarding its effect on penetration of picloram.

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Marked differences in rates of penetration of herbicides and other chemicals through adaxial and abaxial surfaces of the leaf have been reported (9, 14, 22). In general, penetration is greater through the abaxial surface than through the adaxial surface. A decrease in penetration with increasing age of the leaf has been shown by a number of investigators (5, 22). Foliar sprays of 2,4-D and (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) controlled poplar growth most effectively when applied during the early part of the growing season as soon as the leaves were fully expanded (11). Later treatments were progressively less effective.

In general, an increase in temperature, within physiological limits, has resulted in an increase in foliar uptake of herbicides (19, 20, 22). Sargent and Blackman (22) observed that penetration of 2,4-D through the adaxial surface of bean (*Phaseolus vulgaris* L.) leaf discs increased rapidly with increasing temperatures from 3 to 37 C. A change in temperature at constant humidity had less effect on the absorption rate of 1,2-dihydro-3,6-pyridazinedione (MH) than did a change in humidity at constant temperature (26). Higher temperatures accompanied by high humidity were most effective in promoting penetration (19).

Removal of surface waxes from the leaf cuticle increased the rate and extent of cuticular penetration (3, 8). Bukovac and Norris (3) concluded that sorption of napthaleneacetic acid by isolated cuticle of pear (*Pyrus communis* L.) leaf increased on removal of surface wax. Only small further increases resulted from subsequent extraction of embedded waxes.

In the work reported here, an attempt was made to assess quantitatively the influence of added surfactant, stage of plant growth, adaxial or abaxial leaf surface, relative humidity, temperature, and the removal of cuticular waxes, on penetration of picloram and 2,4-D in aspen poplar and balsam poplar.

MATERIALS AND METHODS

Experiments were conducted with detached leaves of aspen poplar collected from mature trees (south bank of the North Saskatchewan River in Edmonton) or from young greenhouse-grown trees. Leaves were collected from outdoor trees in 1967 during June, July, August, and September. Initial bud opening occurred near May 10 and the leaves attained maximum size in about 1.5 months. To minimize variability, the leaves were collected from 1-year-old branches of four selected trees throughout the investigation. Leaves were collected between 8 and 9 AM on bright, sunny days with outside temperatures ranging from 24 to 29 C. The detached leaves were kept turgid during the treatment periods by immersing their petioles in tap water.

In the greenhouse, aspen poplar plants were vegetatively propagated by a technique adapted from Eliasson (10). Root cuttings about 15 cm long were planted in wooden flats with moist vermiculite. When the shoots produced by the root cuttings were 10 to 12 cm high, they were transplanted to 15-cm plastic pots and grown in a conventional greenhouse. Leaves were collected when the plants were 45 to 60 cm tall.

The herbicides included were derivatives of picloram and 2,4-D. Commercial formulations used were potassium salt of picloram, dimethylamine of 2,4-D, and ethyl ester of 2,4-D. Atlox 210, a non-ionic blended surfactant containing polysorbate, mono- and diglycerides, butylated hydroxyanisole, butylated hydroxytoluene, and propylene glycol in a water-isopropanol medium, was added to some herbicide solutions, at a final concentration of 1%.

High relative humidity was attained in a closed system allowed to become saturated or nearly so with water vapor. Most of the experiments were conducted under normal laboratory conditions at temperatures of 25.5 ± 1.5 C in diffuse daylight supplemented by fluorescent light. Low temperatures (10 C) and high temperatures (40.5 C), whenever required, were attained in a cold room and an incubator. Waxes were removed from the leaf surfaces in some experiments by dipping the leaves in chloroform for periods varying from 10 to 240 sec.

Three leaves were used for each treatment. All applications were made in water solution or emulsion. Picloram and the dimethylamine of 2,4-D were applied with a microsyringe, in doses of 0.2 mg in 40 µl of solution. Twenty-µl droplets were placed on the leaves, about 1 cm away from the midvein. The dose of ethyl ester of 2,4-D was 0.1 mg in 40 µl of emulsion. Droplets of emulsion were covered with plastic caps sealed to the leaf with lanolin to minimize volatility losses. Evaporation losses from parafilm were estimated by determining residual amounts after varying intervals.

The amount of residual picloram and 2,4-D on the leaves was determined spectrophotometrically as described by Bandurski (1) for 2,4-D. Absorbance at 270 and 283 mµ for picloram and 2,4-D, respectively, was directly proportional to concentration over a range of 2 to 80 mg/L. One to 24 hr after treatment, the leaves were washed with 5 ml of 50% ethanol in a slow continuous flow from a burette. The concentration of herbicide in the washings then was determined in an aliquot of about 3.5 ml in a Beckman DK Recording spectrophotometer. The difference between the dose applied and the residual amount of herbicide, after correction for volatility losses (negligible for picloram and the dimethylamine of 2,4-D), was assumed to be the amount that had penetrated into the leaf.

The extent of penetration into the leaves also was investigated in some instances by determining the radioactivity in extracts of leaves treated with labeled herbicides. Four 10-µl droplets of water containing a total of 0.1 µc ^{14}C-carboxyl labeled potassium salt of picloram (specific activity 1.03 mc/mmole) were placed on leaves in the manner described previously. Butoxyethanol ester of ^{14}C-carboxyl labeled 2,4-D (specific activity 1.0 mc/mmole) in 50% ethanol was applied to the leaves in two 20-µl droplets containing a total of 0.07 µc 2,4-D (0.53 µg/µl). The treated leaves were washed with 10 ml of 50% ethanol after periods of 1 to 48 hr. The leaves then were ground for 3 min in about 30 ml of 95% ethanol in a Waring blender. The ground material was kept at room temperature for 6 hr, then filtered through Whatman No. 1 filter paper in a Buchner funnel, and filtrates were concentrated to 5 ml under reduced pressure. Aliquots of 0.5 ml were placed on aluminum planchets, dried on a hot plate, and counted in a gas flow counter. Counts were corrected for background; self-absorption was considered negligible.

Foliar penetration and distribution of picloram-^{14}C in leaves was studied in some cases by autoradiography as described by Crafts and Yamaguchi (6). Duplicate picloram-treated leaves were freeze-dried and autoradiographed for 3 weeks using Ansco non-screen safety X-ray film.

Results and Discussion

Influence of added surfactant and relative humidity.

Adaxial surface. Leaves for this series of experiments were collected 25 days after initial bud opening. Results of leaf surface residue determinations 1 to 24 hr after droplet application indicated that surfactant markedly increased the penetration of both picloram and the dimethylamine of 2,4-D (Figure 1). The addition of surfactant resulted in approximately doubling the amount of herbicide penetrated in 24 hr in all cases except for the dimethylamine of 2,4-D under high humidity where the increase was only 61%. The addition of surfactant was more effective under low humidity than under high humidity for both picloram and the dimethylamine of 2,4-D.

Jansen (17) postulated that one of the primary influences of surfactant is the promotion of hydration and swelling of the cuticle under adverse humidity conditions. Our observation that surfactant prevented or postponed crystallization of the herbicide from the treatment droplets during the experiment is in accord with the conclusions of Hughes and Freed (16) and Sargent (21), who considered that surfactants also may act as humecants by retarding drying of the solution, thus providing a longer period of penetration.

Penetration of picloram and the dimethylamine of 2,4-D was much greater under high humidity than under low humidity conditions. This response to high humidity was greater in the absence than in the presence of added surfactant. Relative increases in penetration were greater for picloram than for the dimethylamine of 2,4-D, especially in the presence of surfactant. Under low humidity, the droplets of herbicide solution dried to a white crystalline powder on the surface of the treated spots within 2 hr after application. The prolonged liquid contact under high humidity undoubtedly played a role in bringing about increased penetration. In experiments with radioactive material, very little picloram-^{14}C penetrated under low humidity without added surfactant. Re-wetting the treated spots with 10-µl droplets of distilled water at hourly intervals until 8 hr after initial treatment, under low humidity, brought about a substantial increase in penetration (from 0.2% or less to 3.0% of the dose at 24 hr), but penetration still remained well below that achieved in the same period under high humidity without rewetting (5.6% of the dose applied). Increased pene-

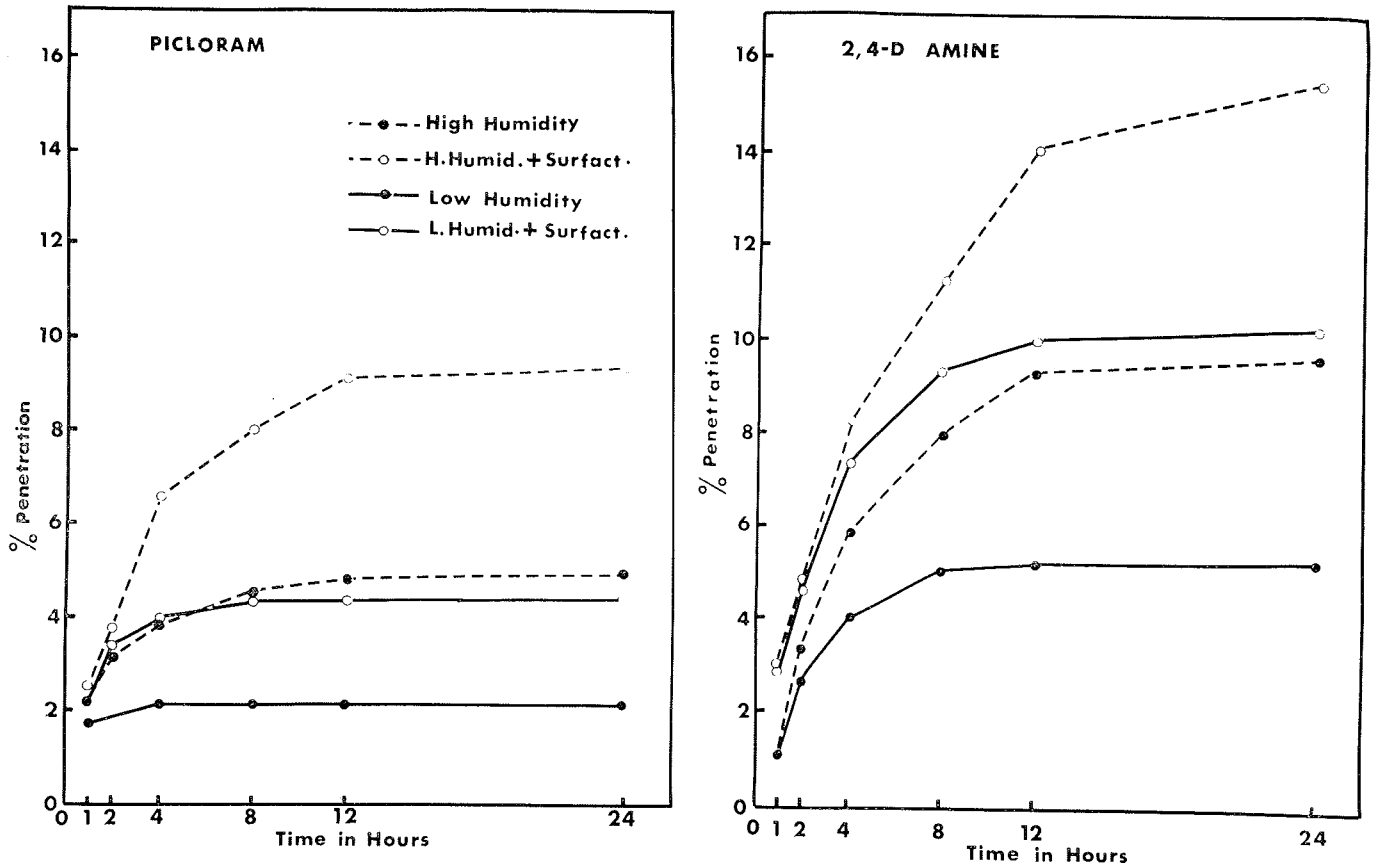

Figure 1. Penetration of picloram and the dimethylamine of 2,4-D, with and without added surfactant (1% Atlox 210), as a percentage of the dose applied (200 μg), from the adaxial surface of aspen poplar leaves under low and high humidity.

tration under high humidity can be attributed only in part, therefore, to a lower rate of drying of droplets. Our results are in agreement with the findings of Pallas (19) and Prasad *et al.* (20) in similar experiments.

Abaxial surface. Both surfactant and especially high relative humidity markedly increased penetration of picloram and the dimethylamine of 2,4-D from the abaxial leaf surfaces (Figure 2). The effect of high relative humidity was similar to that observed for adaxial surfaces. The effect of surfactant, on the other hand, was much less marked, particularly for the dimethylamine of 2,4-D. In fact, under high relative humidity, the surfactant had no observable effect on penetration of this herbicide.

Stomata were observed only on the abaxial and not on the adaxial surface of aspen poplar leaves. At the time of treatments, these stomata were mostly open. It is possible, therefore, that on the abaxial surface, surfactant and high humidity may enhance the uptake of picloram and 2,4-D by promoting the stomatal component of penetration. Stomata are opened more widely under high humidity than under low humidity (19). If penetration occurs through the stomata, such changes in stomatal opening could account, in part, for increased penetration of picloram and 2,4-D under high humidity. The report (5) that, in general, cuticle from abaxial and adaxial leaf surfaces is equally permeable to herbicides, suggests an important contribution from stomatal entry to abaxial penetration in our experiments. The evidence is incomplete, however, and further experiments with detached cuticles are required before more definite conclusions can be drawn.

Under both low and high humidity, and with and without surfactant, the rate of penetration of picloram from abaxial surfaces was two to four times the rate from adaxial surfaces. For the dimethylamine of 2,4-D the abaxial rate was nearly twice the adaxial rate. These results correspond with reports of similar observations in the literature (14, 22).

Penetration of 2,4-D ester from adaxial and abaxial leaf surfaces. In experiments with the ethyl ester of 2,4-D, 1% surfactant was included in all herbicide preparations in order to obtain stable emulsions. Volatility losses after different intervals were subtracted from apparent penetration values to get the net amount of herbicide penetrated into the leaves. These losses ranged up to 56% of the dose applied over a period of 24 hr.

Penetration of the ethyl ester of 2,4-D did not differ greatly between adaxial and abaxial leaf surfaces or between low and high humidity (Table 1). The similarity between results obtained under low and high relative humidity may be due to the fact that herbicide droplets were covered under both types of conditions.

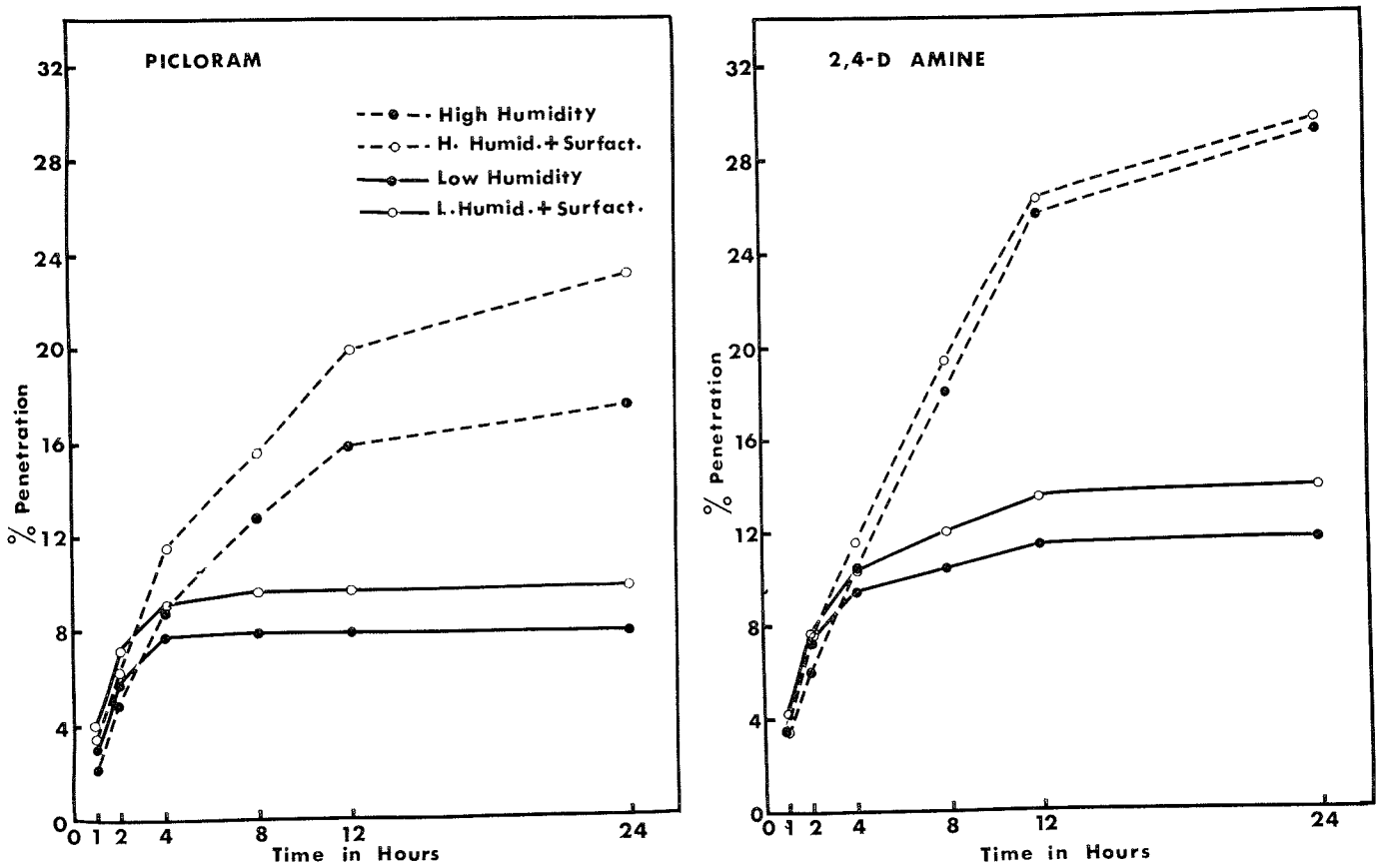

Figure 2. Penetration of picloram and the dimethylamine of 2,4-D, with and without added surfactant (1% Atlox 210), as a percentage of the dose applied (200 μg), from the abaxial surface of aspen poplar leaves under low and high humidity.

Table 1. Penetration of the ethyl ester of 2,4-D, as a percentage of the dose applied (100 μg in 40 μl), from adaxial and abaxial surfaces of aspen poplar leaves under low and high humidity.

Time in hr	Adaxial surface		Abaxial surface		Means
	Low humidity	High humidity	Low humidity	High humidity	
2	5.7	6.2	7.9	8.3	7.0
4	15.7	16.8	18.2	18.3	17.2
8	25.3	25.3	27.6	27.7	26.5
12	31.4	31.4	33.8	33.8	32.6
24	32.8	33.6	33.8	34.6	33.7
Means	22.2	22.7	24.3	24.5	
	L.S.D. 5% = 2.3				

Table 2. Pentration of the ^{14}C-labeled butoxyethanol ester of 2,4-D, as a percentage of the dose applied (0.07 μc in 40 μl), with and without added surfactant (1%), from the adaxial surface of aspen poplar leaves under low humidity.

Time in hr	Without surfactant	With surfactant	Means
2	5.7	6.0	5.8
4	10.6	13.0	11.8
8	21.5	26.5	24.0
12	34.6	42.6	38.6
24	45.9	54.8	50.3
Means	23.7	28.6	
	L.S.D. 5% = 3.2		

The influence of added surfactant on penetration of 2,4-D ester was investigated by using ^{14}C-labeled butoxyethanol ester of 2,4-D. Surfactant under low humidity enhanced penetration of this material from adaxial surfaces of the leaf by about 20% in 24 hr (Table 2). Surfactant enhancement of penetration in this instance was much less, therefore, than that obtained with picloram and the dimethylamine of 2,4-D. This lower surfactant effect may be attributed in part to the presence of ethanol in the herbicide preparation. It is probably more directly related, however, to the non-polar nature of the esters of 2,4-D, as compared with the polar molecules of picloram and the dimethylamine of 2,4-D. The relatively ready cuticular penetration of the non-polar esters of 2,4-D also would account for the similarity in penetration rates of this herbicide through stomata-bearing abaxial and stomata-free adaxial surfaces of aspen poplar leaves.

Influence of stage of growth. Penetration of picloram and the dimethylamine of 2,4-D from adaxial leaf surfaces increased in July and then decreased again in August and September to a level equal to or less than that in June (Table 3). Penetration of the ethyl ester of 2,4-D was influenced but little by growth stage. Our results on the influence of growth stage do not correspond with the results of earlier investigations (11, 22) in which penetration was reported to decrease as the leaf matured. The increase in penetration observed in July in our studies may be attributed in part to certain physical changes observed on the adaxial surface of the leaves. In contrast

Table 3. Penetration of picloram and the dimethylamine and ethyl ester of 2,4-D, as a percentage of the dose applied, with added surfactant (1%), from the adaxial and abaxial surfaces of aspen poplar leaves of different ages, under high humidity during a 24-hr period after treatment.

Growth periods	Picloram		2,4-D amine		2,4-D ester		Means
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	
June	9.4	23.2	15.6	31.5	33.6	34.6	24.6
July	15.1	23.4	26.9	32.1	33.5	32.1	27.2
August	9.4	20.9	14.3	29.5	32.7	31.7	23.1
September	8.8	19.4	14.0	27.6	32.0	32.6	22.4
Means	10.7	20.2	17.7	30.2	32.9	32.7	
			L.S.D. 5% = 3.7				

Table 4. Penetration of picloram and 2,4-D amine with added surfactant (1%), as a percentage of the dose applied (200 μg in 40 μl), 12 hr after application to the adaxial and abaxial surfaces of aspen poplar leaves at three temperatures.

Relative humidity	Temperature (C)	Picloram		2,4-D amine		Means
		Adaxial	Abaxial	Adaxial	Abaxial	
Low	10	3.8	6.4	5.7	7.6	5.9
	25.5	9.5	9.9	11.7	13.9	11.2
	40.5	15.2	15.8	21.2	21.4	18.4
High	10	6.6	8.0	7.3	10.7	8.1
	25.5	15.0	20.6	25.6	26.5	21.9
	40.5	35.0	35.4	44.8	45.6	40.2
Means		14.2	16.0	19.4	20.9	
		L.S.D. 5% = 4.1				

to the smooth and uniform surface in June, the leaf surface 1 month later had many cracks and punctures. These might have developed as a result of changes in the environment (18) or of attack by insects and diseases. Mechanical damage of the leaf surfaces also has been reported to increase permeability to herbicides (5, 15). Dalrymple and Basler (7) observed an increase in absorption of 2,4,5-T in blackjack oak (*Quercus marilandica* Muenchh.) in July over that in June, followed by a decrease in August and September. Although these authors did not offer an explanation of the seasonal variation observed, it is quite likely that physical changes in leaf surfaces as the season progressed were contributing factors. The decrease in penetration in August and September in our studies may be due to the recovery of the leaf surface from such mechanical damage or to increased cuticle thickness with age. Leaves had a glazed appearance in August and September. Juniper (18) stressed that some species that are susceptible to mechanical damage to their wax covering also seem to possess powers of recovery during at least the early stages of their development.

Penetration of herbicides from abaxial leaf surfaces did not differ greatly between June and July, chiefly because the leaves were collected near the ends of both months, and in both instances showed surface damage consisting of small yellowish punctures. In August and September, there was a small decrease in penetration.

The enhancing effect of added surfactant and high relative humidity was similar at all growth stages examined.

Influence of temperature. Penetration of picloram and the dimethylamine of 2,4-D in aspen poplar leaves collected outdoors in the middle of July was examined at 10, 25.5, and 40.5 C, with and without added surfactant, and under both low and high humidity. Treatment periods ranged from 1 to 12 hr.

An increase in temperature resulted in marked increases in penetration of both picloram and 2,4-D under both low and high humidity (Table 4). Mean Q_{15} values over the 10 to 25.5 C range were 2.2 and 2.4 for picloram and 2,4-D, respectively. Corresponding values for the 25.5 to 40.5 C range were 1.8 and 1.7. Values of Q_{15} under high relative humidity were consistently higher than those under low relative humidity, especially in the lower temperature range. Penetration of both herbicides was greater from abaxial surfaces than from adaxial surfaces of the leaf at 10 and 25.5 C, but this difference no longer occurred at 40.5 C. Thus, the influence of increasing temperature in enhancing penetration was more pronounced on adaxial surfaces than on abaxial surfaces of the leaf. High humidity and high temperature together were most effective in bringing about rapid penetration of the herbicides.

Similar experiments with picloram-^{14}C on the adaxial surface, under high relative humidity, using both aspen and balsam poplar leaves, yielded Q_{15} values of 2.2 to 3.3 in the 10 to 25.5 C range, and 5.5 to 10.2 in the 25.5 to 40.5 C range. These results are nearly identical to those obtained with unlabeled picloram, at least in the lower temperature range. In the upper range, they show considerable divergence. We have no satisfactory explanation for this divergence.

The temperature effect probably is due chiefly to physical changes in the cuticular barrier (24), but the possibility of indirect effects through metabolic control of certain steps in the penetration process (21, 22) or through bringing about a steeper concentration gradient in the tissue is not ruled out. Autoradiographs of aspen poplar leaves treated with picloram-^{14}C with 1% surfactant, under high relative humidity, indicated much greater penetration and much more rapid movement within the leaf at 40.5 C than at 25.5 C (Figure 3). These observations suggested at least the possibility that rapid movement of herbicide away from the site of entry, as brought about by an increase in temperature, may be a contributing factor in controlling the rate of penetration.

Influence of chloroform pretreatment. To investigate the role of cuticular waxes in the permeability of adaxial leaf surfaces, some leaves were dipped in chloroform, a good organic solvent for cuticular waxes (8). Such leaves then were treated with picloram and dimethylamine of 2,4-D, without and with added surfactant, and penetration rates over a period of 8 hr were determined.

Prior dipping of the leaves in chloroform increased penetration of both picloram and 2,4-D by about one and one-half to four-fold, depending upon the time of dipping (Figure 4). Dipping periods of 40 to 60 sec resulted in the greatest increases in penetration. Longer periods of dipping which, in addition to removing the surface waxes may begin to extract the embedded waxes, resulted in only small further increases. Added surfactant had little effect on penetration of 2,4-D, and moderately increased that of picloram. These results correspond with literature reports on experiments with isolated cuticles (3, 8), and emphasize the importance of cuticular waxes as barriers to herbicide entry.

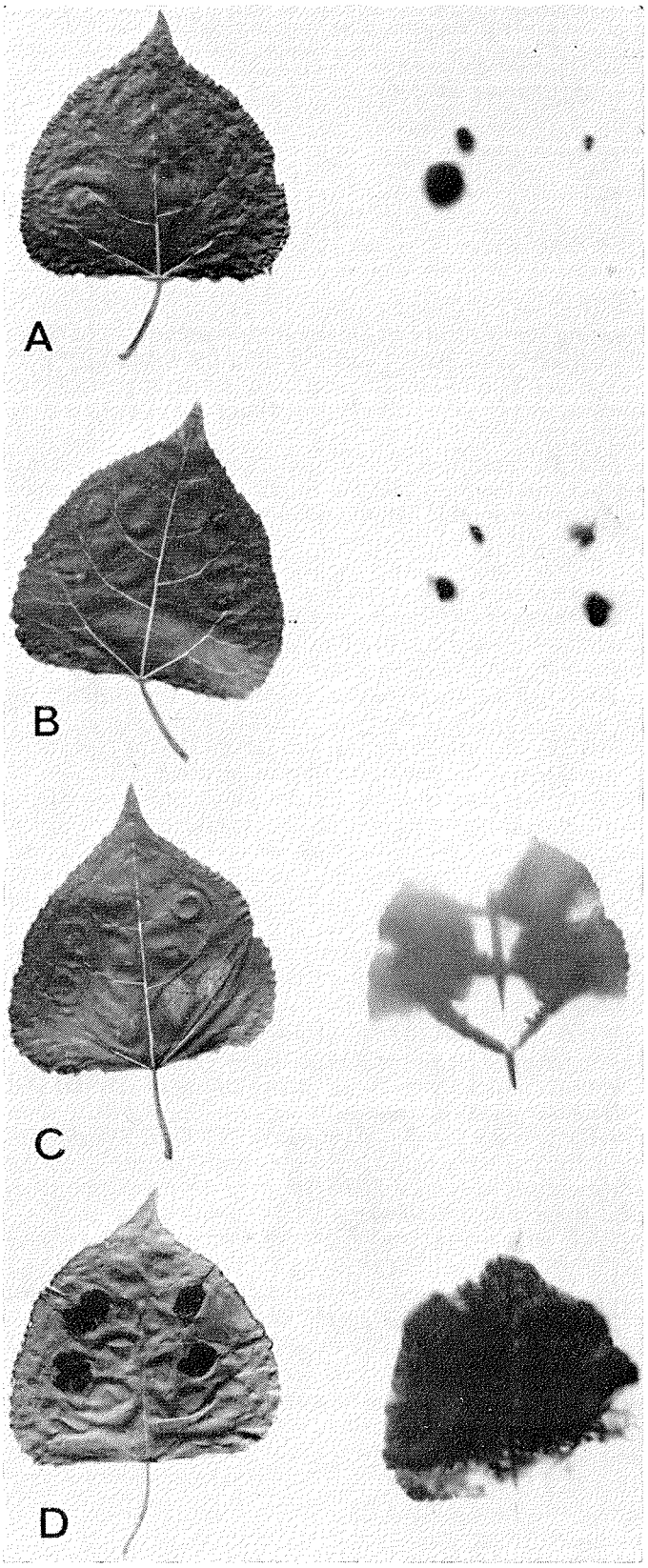

Figure 3. Radioautographs (right) showing penetration and movement of ^{14}C-picloram (0.1 μc in 40 μl), with added surfactant (1% Atlox 210), in aspen poplar leaves treated on the adaxial surface, at 25.5 C (A and B) and 40.5 C (C and D), under high humidity. A. After 24 hr; B. After 48 hr; C. After 8 hr; D. After 12 hr.

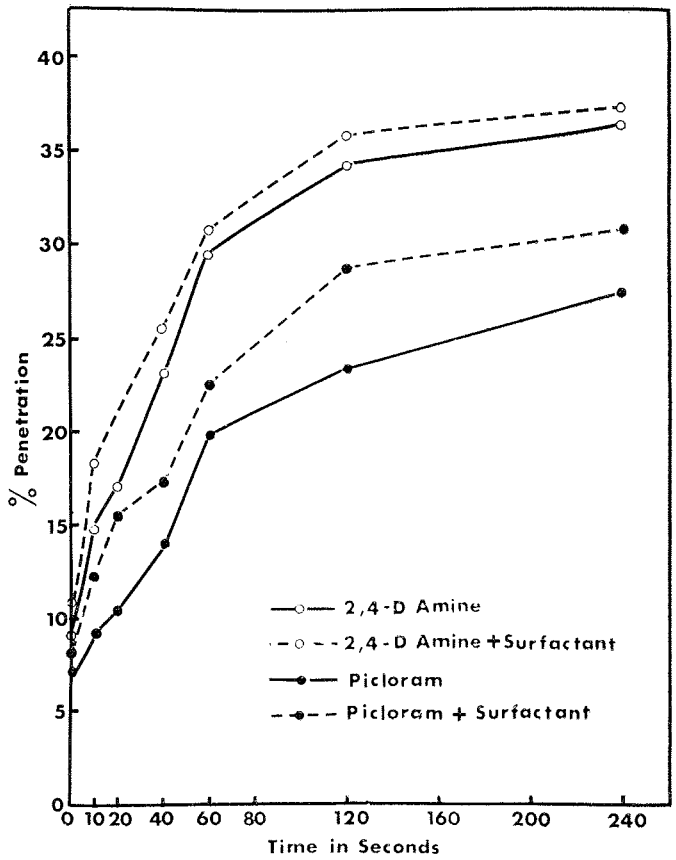

Figure 4. Penetration of picloram and the dimethylamine of 2,4-D, with and without added surfactant (1% Atlox 210), as a percentage of the dose applied (200 μg), after 8 hr of treatment, under high humidity, from the adaxial surface of aspen poplar leaves following dipping in chloroform for varying periods.

Penetration of picloram in balsam poplar. Earlier studies (4) showed that balsam poplar is more resistant than aspen poplar to picloram. We wished to ascertain whether differences in penetration could account for this differential susceptibility. Experiments were conducted with picloram-^{14}C, using detached leaves from greenhouse-grown saplings. The presence of variable amounts of UV-absorbing, ethanol-soluble gummy material on the adaxial surface of the leaves made spectrophotometric determination of residual herbicide impossible.

At 25.5 C, under high relative humidity, picloram entered through the adaxial surface of balsam poplar leaves nearly twice as rapidly as it entered through the corresponding surface of aspen poplar leaves. At 10 and 40.5 C, the difference between the two species was only slightly less marked; penetration in balsam was consistently 1.1 to 1.6 times that in aspen poplar. These observations clearly indicate that differences in penetration of picloram between aspen and balsam poplar cannot account for the reported differences in susceptibility to picloram between the two species.

ACKNOWLEDGMENTS

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